Membrane Receptors & Signal Transduction I

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Retinal Changes Conformation during the Early Stages of Rhodopsin Activation

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G protein-coupled receptors (GPCRs) are integral membrane proteins that account for one third of drug targets. Although they are well studied biophysically, the details of their activation mechanism remain unknown. Here we combine solid-state 2H NMR and molecular dynamics simulations to study activation of the prototypical GPCR rhodopsin in a membrane environment. Our NMR data showed that rhodopsin's ligand, retinal, changed conformations as the protein transitioned between the dark-state and the Meta-I intermediate. To better understand ligand dynamics, we conducted three separate all-atom simulations of rhodopsin in explicit solvent, totaling 4.5 microseconds. These simulations examined dark-adapted rhodopsin bound to 11-cis retinal and two separate simulations tracking the formation of Meta-I. The Meta-I simulations began with a dark-state structure bound to the activating ligand, all-trans retinal. We introduced minor differences between these two simulations to track the role of two glutamates in Meta-I formation. We then computed a set of experimental observables, the deuterium line-shape for individual methyl groups in retinal, from each simulation. The results showed that the dark-state simulation and NMR data matched each other but not Meta-I. Further, the spectra of both Meta-I simulations differed from the dark-adapted NMR spectra, but only one of these matched the experimental Meta-I NMR spectra. The matching simulation exhibited concerted motion between retinal and its Schiff base-linked Lysine-296, and the highly conserved Triptophan-265, distinguishing it from the dark-state simulation.

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On the Relative Stability of Dimeric Interfaces of the Mu-Opioid Receptor Inferred from Recent Crystallographic Studies

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The mu-opioid (MOP) receptor is the predominant target for most clinically used analgesics. In response to demonstrated correlations between opioid dependence and the MOP receptor, researchers have focused on alternative targets. Opioid receptor oligomers are among those that have been suggested to mediate analgesia without the common opioid-related adverse effects. Thus, obtaining a molecular-level understanding of the nature of receptor-receptor interactions in the membrane, either within, or between receptor subtypes, can both create new opportunities for drug discovery, and address the role of oligomerization in receptor function. The recent MOP receptor crystal structure has inspired hypotheses of dimerization contacts, specifically: a closely packed interface involving transmembrane (TM) helices TM5 and TM6, and a less compact one involving TM1, TM2, and helix 8 (H8). These interfaces exhibit similar arrangements to those found in crystals of the chemokine receptor CXCR4 and the kappa-opioid receptor, respectively. While it is tempting to speculate that the tighter TM5/TM6 arrangement has physiological relevance, additional studies are necessary to a) understand the relative dimer stability at TM5/TM6, TM1/TM2/H8, and other interfaces in the membrane, b) evaluate the contribution of the engineered T4 lysozyme (T4L) to the TM5/TM6 association, and c) investigate possible variability across different receptors. To begin to address these questions we have performed umbrella sampling molecular dynamics simulations of coarse-grained representations of the MOP receptor interacting at the TM5/TM6 (with and without T4L) or TM1/TM2/H8 interfaces in an explicit lipid-water environment. We have derived relative estimates of the dimerization free energy at the two specific interfaces and from these we suggest relative dimer lifetimes and dimeric fractions in a lipid mimetic environment. This information can help design future experiments aimed at understanding the role of dimerization in receptor function.

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A Computational Investigation of the Effect of Membrane Curvature on G-Protein Coupled Receptor Oligomerization

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Typical cellular membranes display wide structural and rheological diversity. Such properties have been suggested to impact the spatial organization of membrane proteins with consequent functional implications. Indeed, oligomers of G protein-coupled receptors (GPCRs) are actively investigated for their role in cell physiology and as promising new targets of improved clinical relevance. Inspired by recent experimental findings, we used microsecond-scale molecular dynamics (MD) simulations to investigate the effect of membrane curvature on the mobility and spatial organization of coarse-grained (CG) representations of the prototypical GPCR beta2 adrenergic receptor (B2AR) in a mixture of 80% dioleoylphosphatidylcholine (DOPC), 10% dioleoylphosphatidylglycerol (DOPG), and 10% cholesterol used as a membrane mimetic. Specifically, we explored the self-association of 16 CG B2AR molecules in either a flat lipid bilayer or a closed vesicle with a ~32 nm diameter at a protein/lipid-cholesterol ratio of ~1:450. All simulations were performed with the GROMACS simulation package using the 4 atoms-to-1 bead MARTINI force field. In agreement with simple hydrodynamic models, we find that receptor mobility is hindered at large curvature. A detailed analysis of receptor collisions and residence time of dimeric complexes during simulations yields results consistent with an increased, more stable self-association of receptors in the planar bilayer compared to the small vesicle system.

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Retinal Conformation Governs pKa of Protonated Schiff Base in Rhodopsin Activation

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We have explored the relationship between conformational energetics and the protonation state of the Schiff base in retinal, the covalently bound ligand responsible for activating the G protein-coupled receptor rhodopsin, using quantum chemical calculations. Guided by experimental structural determinations and large-scale molecular simulations on this system, we examined rotation about each bond in the retinal polyene chain, for both the protonated and deprotonated states that represent the dark and photoactivated states, respectively. Particular attention was paid to the torsional degrees of freedom that determine the shape of the molecule, and hence its interactions with the protein binding pocket. While most torsional degrees of freedom in retinal are characterized by large energetic barriers that minimize structural fluctuations under physiological temperatures, the C6-C7 dihedral defining the relative orientation of the β-ionone ring to the polyene chain has both modest barrier heights, and a torsional energy surface that changes dramatically with protonation of the Schiff base. This coupling between conformational degrees of freedom and protonation state is further quantified by calculations of the pKa as a function of the C6-C7 dihedral angle. Notably, pKa shifts of greater than two units arise from torsional fluctuations observed in molecular dynamics simulations of the full ligand-protein-membrane system, implying significant changes in the acidity of the Schiff base prior to forming the activated MII state. These new results shed light on important mechanistic aspects of retinal conformational changes that are involved in the activation of rhodopsin.

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Fluorescence Studies of the Bradykinin 2 and μ-Opioid Receptors Suggest that Caveolae Localization of GPCRs is Mediated by their Attached G Proteins

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Caveolae are 50-100 nm plasma membrane invaginations that have several proposed functions. Some studies suggest that caveolae may influence cell signaling by sequestering certain signaling proteins such as G-protein coupled receptors. We have previously found that Caveolin-1, the main structural protein of caveolae specifically binds to Gαq and that their binding is strengthened upon Gαq activation. Here, we have used fluorescence methods to determine the effect of caveolae on the functional properties and localization of two G-protein coupled receptors: the bradykinin receptor type 2 (B2R), which is coupled to Gαq, and the μ opioid receptor (μOR), which is coupled to Gαi. While caveolae do not affect cAMP signals mediated by μOR, they prolong Ca^{2+} signals mediated by B2R. In A10 cells, down-regulation of Caveolin-1 ablates the prolonged calcium signal in accord with idea that caveolae binds to Gαq. Immunofluorescence and FRET studies show that a significant fraction of B2R resides at or close to caveolae domains while none or very little μOR resides in caveolae domains. FRET between B2R and caveolae is reduced by down-regulation of Gαq or by addition of a peptide that interferes with Gαq/Caveolin-1 interactions suggesting that Gαq promotes localization of B2R to